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(54) Title of the Invention: **A Cosmetic Material Containing Fermented Soybean Extract**

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Specification

1. Title of the Invention

**A Cosmetic Material Containing Fermented Soybean Extract**

2. Claims

(1) A cosmetic material characterized in that it is obtained by the following processes: Fermented soybeans are, in advance, sterilized by heating or sterilized with ethylene oxide gas, after which water is added and they are pulverized, the filtrate then being collected, a solvent that is immiscible with water is added and the material is stirred, after which it is allowed to stand in a cold, dark place, being separated and collected in two parts, the aqueous layer part (A), which has separated, and the solvent layer part (B).

During separation and collection, a solvent that is miscible with water is added to the aqueous layer part (A) and the mixture is stirred, after which it is allowed to stand in a cold, dark place, separating into a precipitate layer part (C) and supernatant layer part (D). These two layer parts are collected separately. The precipitate layer part (C) is an extract that is of high viscosity and of which protein is the principal component and a gummy paste-like elastic solid substance (extract originating from layer C) is obtained by removing the solvent and water from it under reduced pressure. The supernatant layer part (D), from which the solvent is completely removed under reduced pressure, is added to an aqueous solution comprised of one of NaCl, KCl or NaSO<sub>4</sub> (sic) and the component that is precipitated by this means is collected. It is next dissolved in strongly alkaline 50% methanol, after which it is filtered and the filtrate is collected and an extract (extract originating from layer D) comprised of a pigment of which the principal component is isoflavone is obtained by concentration under reduced pressure.

Further, the solvent layer part (B), which has been collected separately in advance and which is immiscible with water, is evaporated under reduced pressure, and, after the solvent has been completely removed, is saponified with an alkali, the unsaponified matter is [illegible] by adding ether or n-hexane and then an extract (extract originating from layer B) of which the principal component, a phytosterol, is obtained, the cosmetic material containing, from these respective fermented soybean extracts, the extract originating from layer C alone or both the extract originating from layer B and the extract originating from layer D together with the extract originating from layer C.

- (2) A cosmetic material characterized in that it contains a dry powder that is obtained in the following way. Fermented soybeans are, in advance, sterilized with ethylene oxide gas, after which water is added and the mixture is gently stirred, the string-like viscous substance that is on the surface of the fermented soybeans is peeled off and transferred to the water that was added earlier, the material then being filtered.

Next, an equal quantity of ethanol is added to the filtrate, and, as the mixture is being thoroughly agitated, the aqueous layer that has separated is collected, acetone is used for the extract containing as its principal component protein that was obtained by removing the water by distillation under reduced pressure and the mixture is thoroughly stirred, after which the acetone is removed under reduced pressure.

- (3) A powdered cosmetic material characterized in that it contains a dry powder that is obtained in the following way. Fermented soybeans are, in advance, sterilized with ethylene oxide gas, after which water is added and the mixture is gently stirred, the string-like viscous substance that is on the surface of the fermented soybeans is peeled off and transferred to the water that was added, the material then being filtered.

Next, an equal quantity of ethanol is added to the filtrate, and, as the mixture is being thoroughly agitated, the aqueous layer that has separated is collected, acetone is used for the extract containing as its principal component protein that was obtained by removing the water by distillation under reduced pressure and the mixture is thoroughly stirred, after which the acetone is removed under reduced pressure, the cosmetic material as described in Claim 2 of the Claims being dried at the time of use.

### 3. Detailed Description of the Invention

This invention relates to growing fermented soybean microorganisms (a species of *Bacillus subtilis*, *Aspergillus oryzae*) in evaporated soybeans, using the food "fermented soybean" which is obtained by maturation as the starting raw material, obtaining the extract from it and using it in cosmetic materials and food products.

Fermented soybeans are of high nutritional value and have long been provided as a food in Japan. However, they have the drawback that not everyone enjoys eating them because they generate an unpleasant odor (stench) on long-term storage. Moreover, many people keep away from fermented soybeans because of the string-like viscous matter that is characteristic of them.

For this reason, the inventors first conducted various studies with the intention of eliminating the unpleasant odor from fermented soybeans and making an extract of them from which the nutritional components were not lost so that they could be used as food products. As a result, the extract from the soybeans and the principal component was a string-like viscous substance and found to consist primarily of protein. This was not only excellent from a nutritional standpoint but also had an excellent moisture retaining and lubricating effect. In particular, when it was applied to the skin, it exhibited a smooth lubricating action, for which reason it was not only found to have nutritional value but also to be advantageous when used as a cosmetic material. Accordingly, the inventors conducted further studies on its application to cosmetic materials, research was continued on the relationship of extraction methods to yields and humectant effects and this invention was perfected as described below.

We have not found any other previous instances of examples of using extracts of fermented soybeans in cosmetic materials or examples of using such extracts as food products. Because the extraction method from fermented soybeans in this invention involves a simple procedure, it can be anticipated that new fields for its utilization will be opened up. Even people who have not eaten fermented soybeans could easily anticipate applications other than cosmetic products for example beverages prepared from their extracts, as mixtures with suitable vehicles and other nutritional agents by processing them as granules, tablets or fillings for gelatin capsules.

[Example 1]

Fermented soybeans were, in advance, sterilized by heating, water was added and they were pulverized to form a gruel-like substance. Next, the gruel like substance was filtered and the filtrate was collected. This filtrate was a viscous liquid. Any one solvent selected, for example, from chloroform, esters such as isoamyl acetate, isopropyl acetate and isobutyl acetate, alcohols such as n-hexyl alcohol and decyl alcohol and hydrocarbons such as n-hexane, n-heptane, benzene, petroleum ether and cyclohexane, which are known solvents immiscible with water, was added in a proportion on the order of 10 to 50% to the filtrate and the mixture was allowed to stand for about a full day in a cold, dark place as it was being stirred. By this means, it was separated into an aqueous layer part (A) and a solvent layer part (B). The A (layer) was collected and separated, after which one solvent miscible with water selected, for example, from ethanol, methanol, acetone and propyl alcohol was added to layer (A) in an amount on the order of 30 to 90% relative to layer A and the mixture was stirred. After stirring, the mixture was allowed to stand for about a full day in a cold, dark place, with the result that it separated into a precipitate layer (layer C) and a supernatant layer (layer D). Layer C (the precipitate) was separated and collected. This precipitate, an extract, contained as the principal component a protein of high viscosity. This extract was then gradually transformed to a gummy paste-like elastic solid by removing the solvent and water under decreased pressure. When it was used in cosmetic materials and foods, it was used as a suspension (emulsion) dispersed in water.

[Example 2]

Layer (B), which had been separated in the process of Example 1 as described above, was distilled under reduced pressure, with the solvent being completely removed. When this was done, fats and oils remained and the characteristic stench (foul odor) of fermented soybeans was present. They

were saponified with an alkali, after which the unsaponified matter was removed by adding ether or n-hexane and an extract of which the principal component, a phytosterol was obtained. The foul odor was thus eliminated from the substance obtained in this process.

### [Experiment 3]

Layer D, which had been separated in the process of Example 1 as described above, was distilled under reduced pressure, with the solvent being completely removed, and it was added to an aqueous solution prepared with any one of NaCl, KCl or Na<sub>2</sub>SO<sub>4</sub>, the component that was precipitated by this means was separated and collected and then dissolved in weakly alkaline 50% methanol, after which it was filtered, the filtrate was collected and an extract of which the principal component was isoflavone and which was comprised of a pigment component was obtained.

The total yields of the extracts that were obtained in the aforementioned Examples 1 to 3 were on the order of approximately 250 to 300 g from 5 kg of fermented soybeans. Of these, the extract of which protein was the principal component (the extract obtained in Example 1) accounted for most of the yield, or 86 to 90%. The remainder was comprised of the extract of which a phytosterol was the principal component (the extract obtained in Example 2), which accounted for 3 to 6%, and the extract comprised of pigment components of which isoflavone was the principal component, accounted for 1 to 3%.

The extracts obtained in the aforementioned examples can be used independently in cosmetic materials and foods. In particular, the extract of which the principal component is protein that was obtained in Example 1 exhibits a high viscosity of about 50 to 80 cps in a concentrated liquid state. At this viscosity, moisture retention and lubricity are exhibited. Further, in mixed solutions of ethanol and water, it is miscible with the extracts obtained in Examples 2 and 3, with lubricity being increased. Specifically, it does not have a smooth sticky feel and has a humectant effect. Therefore, it can easily be used in cosmetic materials and foods. It also has a good taste. In order to bring about the characteristic body of fermented soybeans, it is preferable to make combined use of the extract of which the principal component is a sterol and of the extract comprised of pigment components of which isoflavone is the principal component, which were obtained in Examples 2 and 3, rather than only the extract of which protein is the principal component.

Basically, when suitable fragrances and refrigerants are added and the materials are diluted with water or ethanol, they can be used as simple toilet water and beverages. When the viscosity number at this time is regulated to the order of 10 to 30 cps on the basis of the quantity of extract added or its content, there is a good slippery feeling

characteristic of fermented soybean extracts. The humectant and slipperiness effect is similar to that of [illegible] gum polysaccharides such as hyaluronic acid. It is characteristic that there is no sticky feeling. In short, the humectant effect differs from that attributable to proteins originating from animals such as collagen and albumin in that a smooth, non-sticky touch is obtained.

Next, the fermented soybeans that were the raw materials in the aforementioned examples were heated and sterilized in advance, after which the extraction method was used. Decreases [antecedant not given-Translator] were found as the time required for heat and sterilization in order to obtain high viscosity extracts was prolonged. For this reason, in order to obtain high viscosity extracts, an additional study was made of the distillation procedure. In short, various studies were conducted of the sterilization process and of the intermediate processes and final process during extraction. In all cases, it was found that workability was difficult due to proliferation of the bacteria and that sterilization in advance of the fermented soybeans, which are the raw materials, was the best method for extraction. Moreover, when sterilization with ethylene oxide gas was studied as a method with which there would be no effect on viscosity in the sterilization procedure and which would be advantageous on an industrial production scale, it was found that, over the course of time, extracts of higher viscosity could be obtained and that yields could be increased.

#### [Example 4]

Amounts of 100 g each of fermented soybeans were introduced into a pack for Hi-zex film sterilization of 28 cm in width and 15 cm in width, ethylene oxide gas was sealed into it and it was allowed to stand for specified times as shown in Table 1, after which bacteriological tests (agar plate dilution method) were performed. After sterilization effectiveness was evaluated, amounts of 1000 ml of purified water were added to fermented soybeans that had been subjected to the bactericidal effects of the ethylene oxide gas. They were then stirred at a temperature of  $15 \pm 1^{\circ}\text{C}$  and were further stirred slowly for 1 hour at a rotation rate of 100 rpm, with a viscous substance material similar to a string-like substance on the surface of the fermented soybeans being the principal material extracted. The viscous liquid that was obtained was filtered (Toyo Filter Paper No. 65) by suction filtration. Next, the same volume of ethyl ether was added to the filtrate and the mixture was thoroughly agitated, after which the aqueous layer that was separated was collected. The water in the aqueous layer was removed by distillation under reduced pressure and a solid substance (extract of which protein was the principal component) was obtained. Next, the solid substance was thoroughly washed with acetone, the acetone was removed under reduced pressure and a dry powder was obtained. The yields were as shown in Table 1. It was found that yields

increased and viscosity also increased over the course of time in the ethylene oxide sterilization treatment. Solubility in water also increased by comparison to substances extracted from fermented soybeans that had been subjected to heat treatment.

Table 1 shows the yields and viscosities of extracts of which the principal components were proteins that were extracted using fermented soybeans as a result of ethylene oxide sterilization. The solubility rate (%) was determined for a transparent solution obtained by weighing out amounts of 1 g of extract (extract obtained by Example 4) of which protein was the principal component, introducing the extract into 250 ml of purified water at 20°C and stirring the mixture for 1 hour at a rotation rate of 300 rpm, with an emulsified protein being formed, after which this liquid was subjected to forced filtration using an 0.8 micron membrane filter. In short, the extracts of which proteins were the principal components that were extracted from the fermented soybeans and that were obtained in Example 1 or Example 4 were thoroughly dispersed in water and appeared as a white or milky brown dispersion. There are extremely few liquids that appear as transparent aqueous solutions, i.e., in which the protein components are completely soluble in water. The solubility rates shown in Table 1 are for these water-soluble proteins. The same is true for viscosity. In short, it was found that the protein component when it is emulsified and dispersed in water constituted the principal component with respect to the characteristic viscosity of fermented soybeans. The upper limit of the highest value of the viscosity exhibited by the dry powder itself of the extract of which protein was the principal component as obtained in Example 4 when it was dispersed in water was on the order of 100 cps. On the average, it showed a peak at 80 to 90 cps. As a result, use of fermented soy beans that have been subjected to sterilization treatment with ethylene oxide gas is not only more bactericidally effective against *Bacillus subtilis* than fermented soybeans subjected to heat treatment, but, at the same time, the amount of viscous material that was extracted from the fermented soybeans also increased as treatment time was prolonged. At the same time, it was ascertained that dispersibility and solubility were increased. The cause of this is believed to be that the ethylene oxide becomes attached to the *Bacillus subtilis*, displaying a bactericidal effect and that it also becomes attached to the fermented soybean protein, for which reasons solubility is increased and there are also increases in yields.

(Table 1) Yields of Proteins When Fermented Soybeans Sterilized with E.O. Were Used

(h)	Bacillus subtilis, ordinary bacteria (number)	Mold, yeast (number)	Yield (%)	Solubility (%)	Viscosity (cps)
Untreated	$10^7/g$	0	2.4	0.04	2.4
12	$10^7/g$	0	3.1	0.08	4.7
24	$3 \times 10^3/g$	0	3.1	0.09	4.5
48	$2 \times 10^4/g$	0	3.3	0.10	4.9
72	$5.6 \times 10^3/g$	0	3.2	0.09	4.7
96	3200/g	0	3.4	0.09	4.6
120	550/g	0	3.3	0.11	5.0
144	108/g	0	3.4	0.10	4.6
168	30/g	0			
240	20/g	0			

We shall now describe the uses of extracts obtained in Examples 1 to 4.

The extracts of which proteins were the principal components that were obtained in Example 1 and 4 can be used independently and do exhibit good taste so that they can be used as foods. They can be added to various processed food products as humectant agents and extracts, of which the principal components are proteins, may be dispersed in water to adjust the viscosities to the order of 1 to 20 cps so that they can be used in cosmetic products and beverages. When the extracts, of which phytosterols are the principal components as obtained in Examples 2 and 3 are added to and thoroughly mixed with the extract containing a pigment component of which isoflavone was the principal component and with the extract of which proteins are the principal components as obtained in Examples 1 and 4, the humectant action is different from that of extracts in which proteins are the principal components. In terms of taste, the characteristic body of the fermented soybeans was increased and the lubricating effect was increased.

We shall now present examples of formulations.

(Reference Examples of Formulations)

(1) Beverage

Solution obtained by dispersing the extract, of which proteins were the principal component, obtained in Example 1 or 4, in water and a small quantity of ethanol and adjusting the viscosity to 50 cps



...	1 to 30%
Lactic acid	0.2
Citric acid	0.9
Sweetening agent	3 - 10
Preservative (paraben agents)	0.1
Fragrance	Suitable quantity
Purified water to make a total quantity of 100.	

## (2) Cosmetic Material (Lotion)

Solution obtained by adding water to and dispersing the extract of which the principal component was protein obtained in Example 1 or 4 and the viscosity of which was adjusted to 30 cps

...	5.0%
Whale tallow	2.0
Beeswax	16.0
Liquid paraffin	46.5
Cetyl alcohol	2.0
Purified water	26.8
Borax	1.0
Fragrance and paraben (methyl)	Suitable quantity

## (3) < Vanishing cream >

Stearic acid	16.0%
Sorbitan monostearate	2.0
Polyoxyethylene sorbitan monostearate	1.5
Extract of which protein was the principal component that was obtained in Example 1 or 4	3.5-4.5
Propylene glycol	10.0
Fragrance and paraben (methyl)	0.2

Purified water to male a total quantity of 100.

## (4) < Toilet Water >

Ethanol	9.0%
Lactic acid	0.2
Citric acid	0.9
Sorbitol	4.0
Fragrance, colorant, preservative	Suitable quantities

Aqueous solution obtained by mixing 0.8%  
 of the extract containing pigment  
 component of which isoflavone was  
 the principal component obtained  
 in Example 3 with 20% of the extract  
 of which protein was the principal  
 component obtained in  
 Example 1 or 4 20.0

Purified water to make a total quantity of 100.

(5) < Cold cream >

Beeswax	10.0%
Gelatin	10.0
Vaseline	15.0
Lanolin	5.0
Liquid paraffin	17.5
Olive oil and rice germ oil	10.0
Extract of which protein is the principal component obtained in Example 1 or 4	3.0
Extract of which the principal component is a phytosterol obtained in Example 2	0.5
Extract of which the principal component is isoflavone obtained in Example 3	0.2
Oryzanol	1.0
Purified water	22.7
Fragrance	1.0
Preservative	0.3

[Humectant action]

Next, we shall consider the humectant action of the protein obtained in Example 1 or 4. It was dispersed in advance in water and a solution was obtained, the viscosity of which was adjusted to the order of 30 cps. This solution was further diluted 20 times to make the test solution. The quantity of water that escaped from the solution was found by the gravimetric method to the point that a constant volume was reached at a relative temperature that had been set using a constant temperature and constant humidity tank. The samples were compared using an aqueous solution containing 5% of sodium pyrrolidone carboxylate. The results, as shown in Figure 1, indicate that they had the same humectant action. On the other hand, the toilet water, as indicated below was made using the solution diluted 20 times that was used in the aforementioned test. This toilet water and toilet water to which nothing was added were used in studies of feel on use. Application tests on the skin were carried out

using 40 women as subjects. The results are shown in Table 2. As can be seen, there was no sticky feel, there was a good smooth touch, there was a superior lubricating effect and a clean feel on use.

(Formulation: Toilet water)

Ethanol	9.0%
Lactic acid	0.2
Citric acid	0.9
Sorbitol	4.0
Dilute solution of extract (viscosity, 3 to 5 cps)	8.0
Fragrance	0.1

Purified water to make a total quantity of 100)

(Table 2) Use Response Tests of Toilet Water Containing Fermented Soybean Extract

	Content	Poor	Ordinary	Fairly good	Good
Transparency [poor legibility - Trans.]	Not added	0	2	32	6
	Added	0	20	19	1
Cleanness of skin	Not added	0	6	31	3
	Added	0	5	14	21
Smooth feel of skin	Not added	17	20	3	0
	Added	0	8	12	20

#### [Safety]

It was presumed that there are no problems of safety associated with extracts based on this invention as the starting raw material is fermented soybean which is supplied as a food. However, for the sake of precaution, the extracts of which protein was the principal component, obtained in Examples 1 and 4, were studied by oral administration in mice. The extracts were dispersed in purified water and solutions were used that were prepared of viscosities of approximately 30 cps. The LD<sub>50</sub> values were less than 40 ml and it was concluded that there were no problems and that the materials were of high safety. In addition, primary irritation tests were performed using the aforementioned solution. Forty-eight hour patch tests were performed with the same 40 women who participated in the use response test described above. No abnormalities such as erythema were found.

A further point to which attention should be drawn is that the extracts of which protein was the principal component that was obtained in Example 1 and Example 4 have both a humectant lubricating action and a tyrosinase activity inhibitory action.

When further interest was drawn to this point and we conducted follow-up tests of the extracts obtained in Examples 1 through 4, these actions were found for all of the extracts that were obtained except for that of Example 2.

Consequently, fermented soybeans are advantageous as substances with which both a humectant lubricating action and a beautifying-whitening action on the skin can be expected. Table 3 shows the results of in vitro studies of the melanin pigment production inhibiting action exhibited by the fermented soybean extracts obtained in Examples 1 through 4 of this invention.

The reaction system in the experiments was comprised of 0.5 ml of L-tyrosine (1.0 mg/ml), 2.0 ml of phosphate buffer solution (pH 6.8), 2.0 ml of distilled water or inhibiting agent solution (extract), 0.05 ml of  $\text{Cu}^{++}$  ions (1% solution) and 1.0 ml of tyrosinase (1 mg/ml). The reaction was allowed to proceed for 60 minutes in a constant temperature tank at 37.5°C. After it was concluded, absorbance at 640 nm was measured with a spectrophotometer and the production rate was calculated. Ascorbic acid was used as the comparison test substance.

(Table 3) Melanin Production Inhibiting Action of Fermented Soybean Extract

Test substance (2% inhibiting agent solution, content in solution)	Inhibition rate (%)
Purified water	0
Vitamin C 0.5	97.5
Extract of which protein is the principal component in Example 1 5.0	62.1
Extract of which phytosterol is the principal component in Example 2 1.0	12.6
Extract of which isoflavone is the principal component in Example 3 0.5	68.0
Example of which protein is the principal component in Example 4 5.0	67.4
Mixture of extracts obtained in Examples 1 to 3 at the yield ratios 5.0	63.6

With the dry powder of the extract obtained in Example 4, dissolved at the time of use, a filling of good feel and lubricating characteristics was obtained.

In short, it is a method in which a dry powder is mixed with a cosmetic base material individually or with another powder and in which the mixture was dissolved separately using an aqueous solution, a known toilet water, emulsion or cream.

As shown below, when a powdered cosmetic material was made and toilet water was used, both were collected on the flat of the hand. The powdered cosmetic material was kneaded with the fingertip and was dissolved, being used in that way.

(Powdered cosmetic material)

- |     |                                                 |           |
|-----|-------------------------------------------------|-----------|
| (1) | Dry powder obtained in Example 4                | 1 - 70 %  |
|     | Vitamin C                                       | 0.3 - 50  |
|     | Purified water to make a total quantity of 100. |           |
| (2) | Dry powder obtained in Example 4                | 90 - 95%  |
|     | CMC or alginic acid                             | 5 - 10    |
| (3) | Extract obtained in Example 2                   | 1 - 2 %   |
|     | Extract obtained in Example 3                   | 0.3 - 1   |
|     | Extract obtained in Example 4                   | 80 - 95   |
|     | Oryzanol (fine powdered product)                | 0.2 - 0.3 |
|     | Vitamin C                                       | 1 - 3     |
| (4) | Dry powder obtained in Example 4                | 95 %      |
|     | Aloe polysaccharide powder                      |           |
|     | (Peragel 200)                                   | 0.3       |
|     | Vitamin C                                       | 3 - 4     |
|     | Oryzanol (finely powdered product)              | 0.6 - 2.7 |

The aforementioned powdered types of cosmetic materials are all of a high degree of hygroscopicity (moisture absorbing capacity), for which reason they should be packaged in hermetically sealed containers for solutions. They may be kept in single batches or divided into packets of 0.1 to 2 g. They can also be compounded in foundations and packs. In this case, they may also be used in combination with silk packs and with low molecular weight peptides of silk. In making cosmetic materials, the extracts obtained in Examples 1 through 4 can be mixed in advance in combinations as desired with formulations of other cosmetic base materials and solutions may be made by dispersing and dissolving them in solvents such as water, water and ethanol or water, ethanol and polyols. This is convenient for compounding them.

On the other hand, in the extraction processes in Examples 1 through 4, the fermented soybeans are used after they have been sterilized in advance by heating or with ethylene oxide gas and the substances that are subjected to the extraction treatment operation can also be extracted using fermented soybeans that have not been sterilized in advance. At this time, in the extraction treatment process, the extraction treatment is performed as far as possible at about 20° or at a lower temperature than that. In particular, extracts of which the principal component is protein are concentrated under reduced pressure to make a powder or are placed in a freeze-drier to make a powder, after which, in the final process, the powder is

subjected to sterilization treatment with ethylene oxide gas, by which means it is rendered sterile.

#### 4. Brief Explanation of the Figure

Figure 1 is a graph showing the humectant action of extracts obtained in Example 1 or Example 4 of which the principal component is protein that were made into aqueous solutions and the viscosities of which were adjusted to the vicinity of approximately 3 to 5 cps..

- 1: Dilute solution of extract of this application
- 2: Aqueous solution containing 5% of sodium pyrrolidone carboxylate.

Applicant: Ichimaru Farkos [phonetic]\* Company, Ltd.  
(Representative) Yutaka Ando [seal affixed]

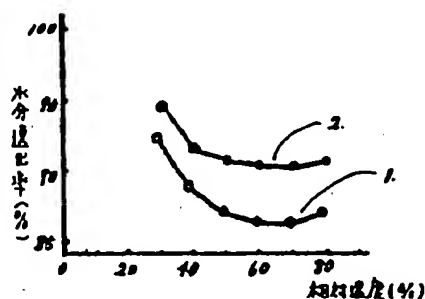


Figure 1

[vertical axis]: Water escape rate (%)

[horizontal axis]: Relative humidity (%)

\*Translator's Note: Transliterated phonetically from the Japanese. As such, the spelling may differ from other transliterations.

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⑭ 納豆抽出物含有化粧料

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⑱ 出 願 昭57(1982)6月23日

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明 細 書

1. 発明の名称

納豆抽出物含有化粧料

2. 特許請求の範囲

(1) 納豆を、あらかじめ加熱滅菌又はエチレンオキサイドガス滅菌させてから、水を加えて溶解させたのち、その濾液をとり、水に混和しないところの溶媒を加えて、攪拌したら、冷暗所に静置させて、分離された水層部(A)と、溶媒層部(B)の二つに分取する。

分取した内、水層部(A)には、水に混和する溶媒を加えて、攪拌後、冷暗所に静置し、沈殿層部(C)と、上澄層部(D)に分層させて、その両層部を別々に取り出す。沈殿層部(C)は、粘性の高い、蛋白質を主体とする抽出物であるが、これを減圧下で溶媒や水分を除去させることによつて、ゴムノリ状の弾力性のある、固型状物(C層由来抽出物)を得る。一方、上澄層部(D)は、減圧下で溶媒を完全に除去させて、NaCl、KCl、Na<sub>2</sub>SO<sub>4</sub>の内、いずれ

かの一種類による水溶液に添加し、これによつて析出した成分を取りだし、次に弱アルカリ性80%メタノールに溶解させてから、濾過して濾液を取り、減圧濃縮により、イソフラボンを主体とする色素成分からなる抽出物(D層由来抽出物)を得る。

さらに、あらかじめ分取した水に混和しない溶媒層部(B)は、これを減圧濃縮して、完全に溶媒を除去したのち、アルカリでケン化させて、その不ケン化物をエーテル又はヨーヘキサンを加えて提取し、フィトステロールを主体とする抽出物(B層由来抽出物)が得られるので、上記の工程で得られた、それぞれの納豆抽出物から、C層由来の抽出物の単独又は、C層由来抽出物と共に、B層由来抽出物、D層由来抽出物の両方又は一方を含有することを特徴とする化粧料。

(2) 納豆をあらかじめエチレンオキサイドガス滅菌してから、水を加えてゆるやかに攪拌し、納豆表面にある、糸引状の粘着物を、剥離さ

せて、先に加えた水に移行させてから、濾過する。

次に、濾液に対して、同量のエタノールを加え、十分に攪混したら、分離した水層部を取りだして、減圧蒸留によつて水分を留去して得られた、蛋白質を主体に含有する抽出物に対し、さらにアセトンを用いて、十分洗淨してから、減圧下でアセトンを留去させて得られた、乾燥粉末を含有することを特徴とする化粧料。

(3) 納豆をあらかじめエチレンオキサイドガス滅菌してから、水を加えてゆるやかに攪拌し、納豆表面にある、糸引状の粘着物を剥離させ、加えた水に移行させてから、濾過する。

次に濾液に対して、同量のエタノールを加え、十分に攪混したら、分離した水層部を取りだして、減圧蒸留によつて、水分を留去して得られた、蛋白質を主体に含有する抽出物に、さら

が、しかし欠点としては、長期間の保存は異臭(クサミ)が発生するために、すべての人々が好んで食べるには至つていなかった。又、納豆特有の糸引状の粘着物は、これもまた敬避する人々も多く、したがつて納豆自体は、嗜好的な側面をもつた食品として、現在に至っている。

そこで、本発明者らは納豆中の異臭を除去し、栄養成分を損うことなく抽出して、これを食品に利用することを当初考え種々の検討を加えた。その結果、納豆中の糸引粘着物を主体に抽出した抽出物は、その主の主体は蛋白質であり、これは栄養的にも優れ、いるばかりか、さらに保湿滑性効果に優れ、とくに肌に塗布すれば、すべすべした潤滑作用を示すことから、栄養供給のみならず、化粧料への利用が有利であることを見出した。そこで、本発明者らは、さらに化粧料への応用に関し、さらに検討を加え、抽出法と収量及び保湿効果との関係について研究を続け、以下に述べるごとく、本発明を完成した。

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にアセトンを用いて、十分洗淨してから、減圧下でアセトンを留去させて得られた、乾燥粉末を含有する、特許請求の範囲、第2項記載の化粧料が、用時蒸留して用いることを特徴とする、粉末化粧料。

### 3 発明の詳細な説明

本発明は高麗大豆に納豆菌(*Bacillus Subtilis* の一種、*Aspergillus Oryzae*)を繁殖させて、熟成して得られる、食用「納豆」をスター原料となし、これをもとに、その抽出物を得て、化粧品類をはじめ、食品類に応用することに関するものである。

納豆は、栄養価の高い食品の一つとして、わが国においては、古くから食用に供されてきた

従来、納豆からの抽出物を化粧料に用いた例や、あるいは食用として抽出物を用いた例は、他に見当らなかつたが、本発明における納豆からの抽出法としては、操作も簡単であるから、新しい利用分野が展開されるものと期待される。すなわち、納豆自体は食べられなかつた人々でも、その抽出物による飲料とか、あるいは顆粒状となし、さらには、錠剤やゼラチンカプセルに充填するなどの加工により、適当な錠形剤や他の栄養剤と混合して用いるなど、化粧品以外においても利用出来やすいものである。

### (実施例1)

納豆を、あらかじめ加熱滅菌し、これに水を加えて液砕し、カユ状物となしたら、次にカユ状物を濾過して、その濾液を得る。この濾液は粘稠な液であるが、次に水に混和しないところの公知な溶媒である、たとえばクロロホルム、



酢酸イソアミル、酢酸イソプロピル、酢酸イソブチルなどのエステル類、 $\gamma$ -ヘキシルアルコール、デシルアルコールなどのアルコール類、 $\gamma$ -ヘキササン、 $\gamma$ -ヘプタン、ベンゼン、石油エーテル、シクロヘキササンなどの炭化水素から適宜選んだ、いずれかの一種の溶媒を、油液に対して10～50%程度を加えて、よく攪拌させてから、一昼夜程度、冷暗所に静置し、これによつて水層部(A層)と油層部(B層)とに分離し、A層を分取した後に、A層に対し水に飽和する溶媒である、たとえば、公知なエタノール、メタノール、アセトン、プロピルアルコールなどから適宜選んだ一種類を、A層に対して50～90%程度を加えて攪拌する。攪拌後は一昼夜程度、冷暗所に静置し、沈殿物層(C層)と上澄液層(D層)に分離し、C層を分取し、沈殿物を取り出す。このものは、粘性の高い蛋白質を主体とする抽出物である。この抽出物は、さらに減圧下で溶媒や水分を除去することにより、次第にゴムノリ状の弾力性のある固形

物を主体とする、色素成分からなる抽出物を得た。

以上の実施例1～3で得られた各抽出物の収量は、納豆3gから約200～300g程度であつた。その内、蛋白質を主体とする抽出物(実施例1で得た抽出物)が、ほとんどであつて、80～90%をしめ、残りはフィトステロールを主体とする抽出物(実施例2で得た抽出物)が3～6%、イソフラゴンを主体とする色素成分からなる抽出物は、1～3%程度であつた。

前記実施例で得られたエキスは、それぞれ単独で、化粧品や食品に用いることも可能であるが、とくに実施例1で得た、蛋白質を主体とする抽出物は、粘度が高く、濃縮した液体状態で30～80 cps前後の値を示す。この粘度は、保湿滑性を示し、又エタノールや水の混液中では、実施例2～3で得られたところの各抽出物とも良く混和し、滑性が高まる。すなわち、すべすべした、べたつき感のない、保湿効果を

有するものが、化粧品や食品に用いるときは、再度、水に分散した懸濁(乳状)物を用いる。

#### (実施例2)

上記した実施例1の工程中で分離したB層を用い、これを減圧蒸留して完全に溶媒を除去すると、油脂類が残留する。このものには、納豆特有のクサミ(臭気)が移行しているも、これをアルカリでケン化させてから、その不ケン化物を、エーテル又は $\gamma$ -ヘキササンを加えて攪取し、フィトステロールを主体とする抽出物を得た。この工程で得たものは、臭気は除去されている。

#### (実施例3)

前記した実施例1の工程中で分離したD層を用い、これを減圧蒸留して完全に溶媒を除去し、 $\text{NaOH}$ 、 $\text{KCl}$ 、 $\text{H}_2\text{SO}_4$ の内、いずれかの一様による水溶液に添加し、これによつて析出された成分を分取し、次いで弱アルカリ性30%メタノールに溶解させてから、濾過して濾液を取りだし、減圧濃縮することによつて、イソフラゴ

ンを主体とする、色素成分からなる抽出物を得た。又、味も良好であり、納豆特有のコクを出すには、蛋白質を主体とする抽出物のみよりも、実施例2や3で得たところのステロールを主体とする抽出物や、イソフラゴンを主体とする色素成分からなる抽出物を併用すると良い。

基本的には、適当な香料や清涼剤を加え、水やエタノールなどにより希釈すれば、簡単な化粧水や、飲料として用いることが出来るも、その際の抽出物の添加量又は含有量としては、粘度数値をもとに、10～30 cps程度に調整すると、納豆抽出物が示す特有の滑性フィリングが良好となる。保湿滑性効果は、ヒアルロン酸などの粘性ムコ多糖類と類似しており、べたつき感がないのが特徴である。つまり、コラーゲンやアルブミンなどのような、動物由来の蛋白質をもとにした保湿効果と異なり、べたつきがなく、さらつとした感触が得られる。

次に、前記の実施例においては、原料である納豆を、あらかじめ加熱滅菌してから抽出する

方法を採用したが、高粘度な抽出物を得るには、加熱減圧に要する時間が長くなるほど、低下することがわかった。そこで、高粘度な抽出物を得るために、減圧操作について、さらに再検討を加えることにした。つまり、減圧操作について、抽出の際の中間工程や、最終工程などにおいて、種々の検討を加えてみたが、いずれも作業性は減圧の増大などにより困難であり、あらかじめ原料となる納豆を減圧させ、これをもとに抽出する方法が最善であることがわかった。そこで、さらに減圧操作について、粘度に影響しないで、工業的生産規模においても有利な方法について、エチレンオキサイドガス減圧を試みたところ、経過時間により、むしろ粘度も高い抽出物が得られると共に、さらに、収量的に増加することがわかった。

#### (実施例4)

納豆各々100gを用い、縦28cm×横13cmのヘイゼックスフィルム減圧用ベックに入れ、エチレンオキサイドガスを第1表に示すこと

向を示した。

第1表は、エチレンオキサイド減圧による、納豆を用いて抽出された、蛋白質を主体とする抽出物の収量及び粘度についてみたものである。尚、溶解率(%)は、蛋白質を主体とする抽出物(実施例4で得られた抽出物)各々1gを精秤し、20mlの精製水250ml中に入れて攪拌し、回転数300rpmで1時間行い、乳濁蛋白質液となした後、この液体を0.8ミクロンのメンブランフィルターにより、強制濾過させて、得られた透明な溶液について測定したものである。つまり、実施例1又は実施例4において得られるところの、納豆から抽出された蛋白質を主体とする抽出物は、水によく分散するも、乳白色又は乳黄色の懸濁液を呈するものであるが、透明な水溶液を呈した液体、すなわち完全に水に可溶性の蛋白質部分は、ごくわずかであり、第1表に示す溶解率は、この水溶性蛋白質について示したものであり、粘度についても、同様に示したものである。つまり、納豆の有する特

く、所定時間封入して放置したのち、減圧試験(準天平板浸漬法)を行い、減圧効果判定したのち、それぞれのエチレンオキサイドガス浸漬時間について、精製水1000mlを加えて、温度18±1℃で攪拌させ、100rpmの回転板で1時間、緩やかに攪拌させ、納豆表面にある未引伏の蛋白質を主体とする、粘稠物を抽出した。得られた粘稠な液を、吸引濾過法により、濾紙(東洋濾紙No.66)で濾過する。次に、濾液に対して、同量のエチルエーテルを加え、十分に振盪してから、分離する水層を振り出して、これを減圧蒸留によつて、水を留去させて固形物(蛋白質を主体とする抽出物)を得る。次に、アセトンで固形物を十分洗浄し、減圧下でアセトンを留去させて、乾性粉末を得る。収量は、それぞれ第1表に示すごとくであつた。エチレンオキサイド減圧処理における時間の経過と共に、収量が増加し、粘度も上昇することがわかった。又、水に対する溶解性も、加熱処理した納豆から抽出したものに比べて高まる傾

向の粘度は、水に乳濁分散するところの蛋白質部分が、その主体をなしていることがわかった。一方、実施例4によつて得られたところの、蛋白質を主体とする抽出物の乾性粉末自体が示す粘度は、これを水に分散させて、一番高い数値の上限は、100cps程度となり、平均的には80〜90cpsにピークを示す。この結果、加熱処理した納豆を用いるよりも、エチレンオキサイドガス減圧処理した納豆を用いる方が、枯草菌に対する殺菌に有効なばかりでなく、同時に処理時間の延長は、これにともなつて納豆から抽出される、粘稠な蛋白質の量も増加することがわかった。同時に水に対する分散性及び溶解性も向上することが判明した。この原因は、エチレンオキサイドが枯草菌に附加して、殺菌効果を表わすと共に、納豆蛋白にも附加して、これがために溶解性が向上し、収量的にも増加を示したものと考えられる。

(第1表) 5・0・数値による納豆を用いた、蛋白質の収量など

例	付草量・一担 量 (g)	カビ・納豆 量 (g)	収率 (%)	納豆率 (%)	粘度 (cps)
未処理	10 <sup>7</sup> /g	0	2.4	0.04	2.4
12	10 <sup>7</sup> /g	0	3.1	0.08	4.7
24	3×10 <sup>5</sup> /g	0	3.1	0.09	4.8
48	2×10 <sup>5</sup> /g	0	3.3	0.10	4.9
72	3.5×10 <sup>5</sup> /g	0	3.8	0.09	4.7
96	3200/g	0	3.4	0.09	4.8
120	530/g	0	3.3	0.11	5.0
144	108/g	0	3.4	0.10	4.8
168	30/g	0			
240	20/g	0			

次に実施例1～4で得られた各々の抽出物について、その用途面に関して述べる。

実施例1及び4で得られた蛋白質を主体とする抽出物は、そのまま単独でも味も良好であり、食用とすることもできる。又、保潔剤としても各食品の加工食品中に添加して用いることも出来るが、化粧品や飲料では、蛋白質を主体とす

香 料 ..... 適 量  
精製水をもつて全量を100とする。

#### (2) 化粧品〈ローション〉

実施例1又は4で得た蛋白質を主体とする抽出物を、水を加えて分散させ、粘度を30 cpsに調整した溶液 ..... 5.0 多  
 鯨 ロ ウ ..... 2.0  
 ミ フ ロ ウ ..... 1 6.0  
 流動パラフィン ..... 4 6.5  
 セチルアルコール ..... 2.0  
 精 製 水 ..... 2 6.8  
 ホ ウ 砂 ..... 1.0  
 香料及びパラベン (メチル) ..... 適 量

#### (3) 〈ベニシニングクリーム〉

ステアリン酸 ..... 1 6.0 多  
 ソルビタン・モノステアレート ... 2.0  
 ポリオキシエチレンソルビタン  
 モノステアレート ..... 1.5

実施例1又は4で得た、蛋白質を主体とする抽出物 ..... 3.5 ~ 4.5

る抽出物を、水などに分散させて、粘度について1～20 cps程度になるように調整して用いると良い。さらに、実施例2～3で得られたところのフィトステロールを主体とする抽出物や、イソフラゴンを主体とする色素成分を含む抽出物とは、実施例1や4で得られたところの蛋白質を主体とする抽出物とは、よく混和するので一緒に添加して用いると、保潔作用は蛋白質を主体とする抽出物と変わらないが、味は納豆特有のコクが増し、滑性効果は向上する。以下に、処方例を示す。

#### (参考処方例)

##### (1) 飲料

実施例1又は4で得た蛋白質を主体とする抽出物を、水と少量のエタノール中で分散させ、粘度を80 cpsに調整した溶液 ..... 1 ~ 30 多  
 乳 液 ..... 0.2  
 クエン酸 ..... 0.9  
 甘 味 料 ..... 3 ~ 10  
 防腐剤 (パラベン類) ..... 0.1

プロピレングリコール ..... 1 0.0  
 香料及びパラベン (メチル) ..... 0.2

精製水をもつて全量を100とする。

##### (4) 〈化粧品〉

エタノール ..... 9.0 多  
 乳 液 ..... 0.2  
 クエン酸 ..... 0.9  
 ソルビト ..... 4.0  
 香料、着色料、防腐剤 ..... 適 量

実施例1又は4で得た蛋白質を主体とする抽出物20多に、実施例3で得たイソフラゴンを主体とする色素成分を含有する抽出物0.8多を混和した水溶液 ..... 2 0.0  
 精製水をもつて、全量を100とする。

##### (5) 〈コールドクリーム〉

ミフロウ ..... 1 0.0 多  
 セレシン ..... 1 0.0  
 ワセリン ..... 1 3.0  
 ラノリン ..... 3.0  
 流動パラフィン ..... 1 7.5

オリーブ油又は米胚芽油	10.0
実施例1又は4で得られた蛋白質を主体とする抽出物	3.0
実施例2で得られたフィトステロールを主体とする抽出物	0.3
実施例3で得られたイソフラボンを主体とする抽出物	0.2
オリザノール	1.0
精製水	22.7
香料	1.0
防腐剤	0.3

## 〔保湿作用〕

次に、実施例1又は4で得られた蛋白質の保湿作用についてみると、あらかじめ水で分散させて、粘度を30 cps程度に調整させた溶液を用い、これをさらに20倍希釈した液を検液とし、低温恒湿槽により、設定した相対湿度下において、検液が逸出する水分量を、恒量に至った時点で重量法によつて求めた。製品としては、ピロリドンカルボン酸ナトリウムの6%含有

水溶液を用いて比較したが、その成績結果は第1図に示すごとく、ほぼ同じ保湿作用を有していることがわかった。一方、前記試験に用いた20倍希釈液を用いて、次に示す化粧水を作り無添加の化粧水と、使用感について、40名の女性を対象にして、肌に対する使用試験を行ったが、その結果は第2表に示すごとく、べたつき感がなく、すべすべした感触が良好で滑性効果に優れ、さつぱりとした使用感が得られた。

## 〔処方：化粧水〕

エタノール	9.0%
乳 酸	0.2
クエン酸	0.9
ソルビト	4.0
抽出物の希釈液（粘度3～5 cps）	8.0
香料	0.1

精製水で全量100とする。

(表2表) 納豆抽出物含有化粧水の使用感テスト

	含有	強い	普通	やや強い	良好
通 明 感	無添加	0	2	32	6
	添加	0	20	19	1
肌のツツベリさ	無添加	0	6	31	3
	添加	0	8	14	21
肌のユブスベさ	無添加	17	20	3	0
	添加	0	8	12	20

## 〔安全性〕

本発明による抽出物における安全性については、そのスタート原料が納豆であり、食用に供されており、何ら問題はないものと推定されたが、念のために、マウスによる経口投与により、実施例1及び4において得られた、蛋白質を主体とする抽出物について、これを精製水で分散させて、粘度を約30 cps附近に調整した溶液をもとに実施したが、LD<sub>50</sub> 値は40 ml以上であり、何ら問題なく安全性が高いものであると評価された。一方、一次刺激性試験につい

ては、前記の溶液をもとに、48時間のパッチテストを、前述した使用感テストと同一の女性40名を対象に実施したが、紅斑などの異状は認められなかった。

さらに、注目されることは、実施例1や実施例4で得られたところの、蛋白質を主体とする抽出物には、保湿滑性作用と共に、チロジナーゼ活性の阻害作用があり、さらに、この点に興味をもつて、実施例1～4で得られた、それぞれの抽出物において、追試を行ったところ、実施例2以外で得られた抽出物には、いずれもその作用があることがわかった。

したがって納豆抽出物は、保湿滑性作用と共に、肌の美白的効果も期待出来るものとして有利なものである。第3表は、本発明の実施例1～4で得られたところの納豆抽出物に示す、メチレン有色々素生成抑制作用について、インビトロにおける成績結果を示したものである。

試験における反応系は、L-チロジン（1.0 mmol/l）0.5 ml、リン酸緩衝液（pH6.8）2.0

ml、蒸留水又は阻害剤液（抽出物）2.0 ml、 $Ca^{++}$ イオン（1%溶液）0.05 ml、チロジナーゼ（1mg/ml）1.0 mlにより、37.5℃の恒温槽中60分の反応を進行させ、終了後に分光光度計640 nmの吸光度を求め、生成率を算出した。比較機体としては、アスコルビン酸（ビタミンC）を用いた。

（第3表）納豆抽出物のメラニン生成抑制作用

機体（阻害剤濃度は、表中の含有量）	阻害率%
機 体 水	0
ビタミンC 0.5	97.8
実施例1による蛋白質主体の抽出物 5.0	42.1
“ 2によるフィトステロール主体の抽出物 1.0	12.8
“ 3によるイソフラボン化合物主体の抽出物 0.5	48.0
“ 4による蛋白質主体の抽出物 2.0	47.4
“ 1～3で得られた抽出物の重量比率で混合したもの 5.0	43.6

さらに、実施例4で得られたところの抽出物の乾燥粉末は、これを用時溶解させて用いると、一段と感度のよい、滑性のあるフィリングが得られることである。

#### アロエ多糖体粉末（ベラゲル-200）

..... 0.5

ビタミンC ..... 3～4

オリザノール（微粉末化品）..... 0.5～2.7

上記した粉末タイプの化粧料は、いずれも吸水性（吸湿性）が高いため、充填容器は密閉した包装が良く、さらに1回分として、0.1～2g程度に分包状態にしておくとうまい。この他、ファンデーション類やパフ類などにも配合出来るが、その際はとくに、シルクパウダーやシルクの低分子化されたペプチドなどとの併用が良い。化粧料向には、他の化粧料基剤との処方上、あらかじめ、実施例1～4で得られた抽出物を、自由な割合で混合し、水又は水とエタノール、あるいは、水とエタノール及びポリオール系などの溶剤中に分散溶解させた液を作り、これを配合することが便利である。

一方、実施例1～4の抽出工程では、あらかじめ加熱又はエチレンオキサイドガスによる、滅菌処理した後の納豆を用いて、抽出処理操作

つまり、乾燥粉末を、単独か又は別の粉末状の化粧料基剤と混合しておき、これとは別に水溶液又は、既知の化粧水や乳液、あるいはクリームなどを持ちいて、溶解させて用いる方法である。以下に示すような、粉末化粧料を作り、化粧水などを使用する際に、手の平などで両方を取り、粉末化粧料を指先などで随分溶解させて用いるものである。

#### （粉末化粧料）

（1）実施例4で得た乾燥粉末 ..... 1～70%

ビタミンC ..... 0.5～10

デンプンをもつて全量100%となす。

（2）実施例4で得た乾燥粉末 ..... 90～95%

0.5%又はアルギン酸 ..... 5～10

（3）実施例2で得られた抽出物 .. 1～2%

“ 3 “ .. 0.5～1

“ 4 “ .. 80～95

オリザノール（微粉末化品）..... 0.2～0.5

ビタミンC ..... 1～5

（4）実施例4で得られた乾燥粉末 ..... 95%

に入っているも、あらかじめ滅菌処理しない納豆を用いて、抽出することも出来る。その際は、抽出処理工程においては、なるべく20℃前後又は、それ以下の温度で抽出処理を行い、とくに、得られた蛋白質を主体とする抽出物については、これを減圧下で濃縮して粉末とするか、凍結乾燥機にかけて、粉末となしたのち、最終工程において、この粉末に対して、エチレンオキサイドガス滅菌処理により、無菌化することが望ましいことがわかった。

#### 4. 図面の簡単な説明

第1図は、実施例1又は4で得られた、納豆から蛋白質を主体とする抽出物の、水溶液としたもので、粘度が約3～5 cpa 附近に調整した状態にあるものの、保湿作用を示すグラフ。

1は、本願抽出物の希釈液

2は、ピロリドンカルボン酸ナトリウムの5%含有水溶液。

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